RESISTIVITY AND PERMITTIVITY CHARACTERISTICS OF HUMAN BLOOD PLASMA DURING COAGULATION

A THESIS

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Approved:

Chairman

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SUMMARY

The object of this thesis was to measure and study the resistivity and permittivity characteristics of human blood plasma during coagulation. It was believed that useful information may be contained in impedance changes of blood plasma which occur during the clot reaction. In sol-gel reactions similar to the clot reaction, the resistivity of the sample changes during the gellation process. If the resistivity of blood also changes, it may be possible to study various parameters of the blood condition such as the clotting time by purely electrical means.

The clotting time is often used by physicians in their diagnosis of circulatory disorders. At the present time, the majority of clotting time measurements are made by mechanical methods. These methods introduce errors into the measurement results because physical agitation inherent within them accelerates the clotting reaction. The measurement of an electrical parameter associated with the blood sample would not introduce agitation and thus might provide a more accurate method for the determination of the clotting time.

An a.c. bridge driven by an 1 kHz generator was employed to measure the resistivity and permittivity of blood plasma. The resistivity of each of the ten samples tested decreased during coagulation. The range of the resistivity changes was between 3 and 9 per cent of the initial value. The permittivity of each of the ten samples changed less than 2.2 per cent of the initial value during the coagulation process.

Analysis of the data revealed that the resistivity of the plasma varied approximately as an exponential with respect to time. Results of an attempt to correlate the clotting time of each sample with the time constant of the associated exponential were inconclusive; however, when the resistivity of the sample ceased to change it was found that the plasma sample had clotted. No clot timing device was developed from the study, but such an approach seemed practical.

CHAPTER I

INTRODUCTION

The Blood Clotting Phenomenon

At the present time, medical treatment of many circulatory diseases often requires the addition of compounds to the blood which either promote or retard the formation of blood clots. Since these drugs upset the complex chemical balance in blood which controls clotting, the effects of these additives on each patient must be followed closely. One of the most widely accepted measures of blood condition is clotting time, which is defined to be the time required for a sample of fresh, whole blood at body temperature (37° C) to coagulate to a consistency such that it will not pour from its container[1]. Normally whole blood clots in four to ten minutes, but the addition of pro- or anti- coagulents may reduce the time to seconds or prevent clotting completely.

Normal blood contains more than ten different compounds which influence the clotting process[2]. The most important of these are thromboplastin, prothrombin, calcium ions, and fibrinogen. Thromboplastin is found in the tissue immediately surrounding the blood vessels. The other three are contained in the blood itself. According to the classical theory, a rupture in a blood vessel allows thromboplastin to enter the blood through the wound and to react with calcium ions and prothrombin to form thrombin. The thrombin causes the fibrinogen to form fibrin, a stringy, sticky substance which catches red blood cells flowing across

it, and creates the clot[3]. This process is illustrated in Figure 1. This last conversion of fibrinogen to fibrin is basically the precipitation of a protein in a liquid (sol) state to the solid (gel) state[4].

Environmental factors as well as blood chemistry influence the clot time. Contact with wettable surfaces such as glass tends to accelerate the clot reaction[5]. Deviation of the sample's temperature from 37° C (body temperature) in either direction changes the clotting time[6]. Physical agitation decreases the clotting time[7].

Present Methods of Clotting Time Measurements

The acquisition of blood for testing requires the addition of some anti-coagulent to the sample during collection. Otherwise, the clot reaction in the sample would begin as soon as the vein from which it is taken was punctured[8]. Usually it is not convenient to begin timing the reaction at this point. Normally, the blood sample is taken in a test tube containing either sodium citrate or sodium oxalate, both of which prevent coagulation by absorbing calcium ions[9]. This mixture is then transported to a laboratory for testing at a later time.

At the present time, medical laboratories frequently use one of three methods to determine clot time:

1. Lee-White Test
2. Fibrometer Timing
3. Thrombelastographic Study

The Lee-White test relies upon the physical observation of the blood sample and the use of a stopwatch to determine the clotting time. To implement the test, the sample is poured into three test tubes immersed in a 37° C water bath. The clot reaction is restarted (usually by adding calcium ions in the form of calcium chloride), the timing device

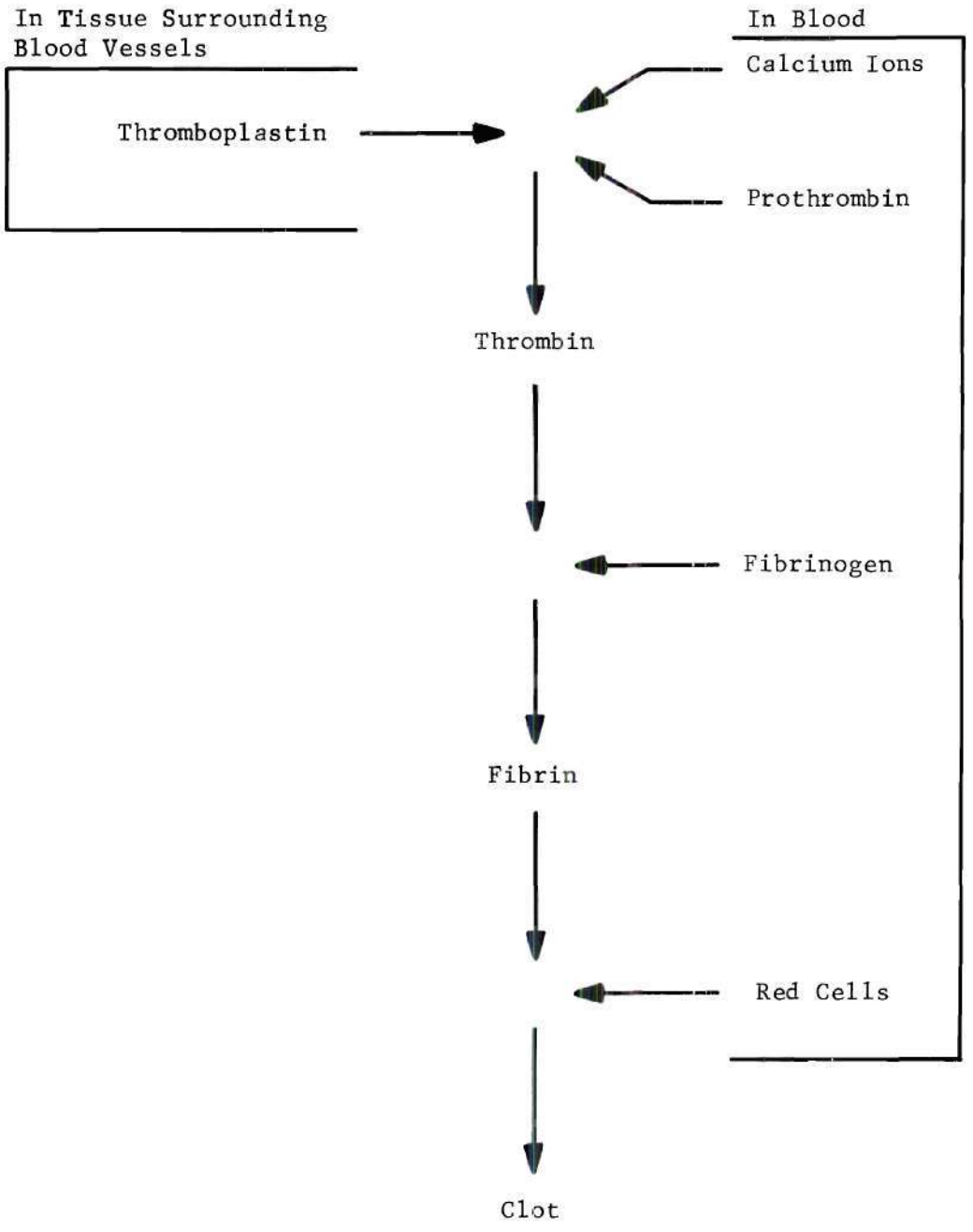


Figure 1. Classical Clot Process.

started, and the first test tube is tipped from side to side at thirty second intervals until the sample does not pour. Since agitation accelerates the clot reaction in the first test tube, the remaining two samples are unclotted. Test tube two is tipped until its contents do not pour. By this time, the contents of test tube three should be on the verge of clotting. When the blood in test tube three will not pour, the timer is stopped, and the elapsed time recorded and taken as the clotting time[10]. In the Lee-White test, the judgement of the person performing the test as to when the blood has reached a certain consistency can cause significant error[11].

There are a number of variations to the Lee-White test in which slightly different chemical treatment is given to whole blood or a blood component such as plasma. Each treatment allows assay of a particular blood component such as fibrinogen, thromboplastin, or vitamin K. However, all variations follow the basic observation and timing technique[12].

The fibrometer is an instrument which automates the Lee-White test. Figure 2 illustrates its construction. Two test probes are immersed in the sample and gently rocked back and forth. When the blood clots, the probes are held together by the solid blood mass, tripping an external alarm. However, small amounts of blood may adhere to each of the contacts and prevent a good low-resistance path between the probes. Even though the blood coagulates, the alarm will not be tripped. In order to determine the clot time, the test must be re-run, possibly several times. Error is introduced into this method by the agitation which decreases the clot time. The principal advantage of the fibrometer is that the probes will always be immobilized when the blood has reached

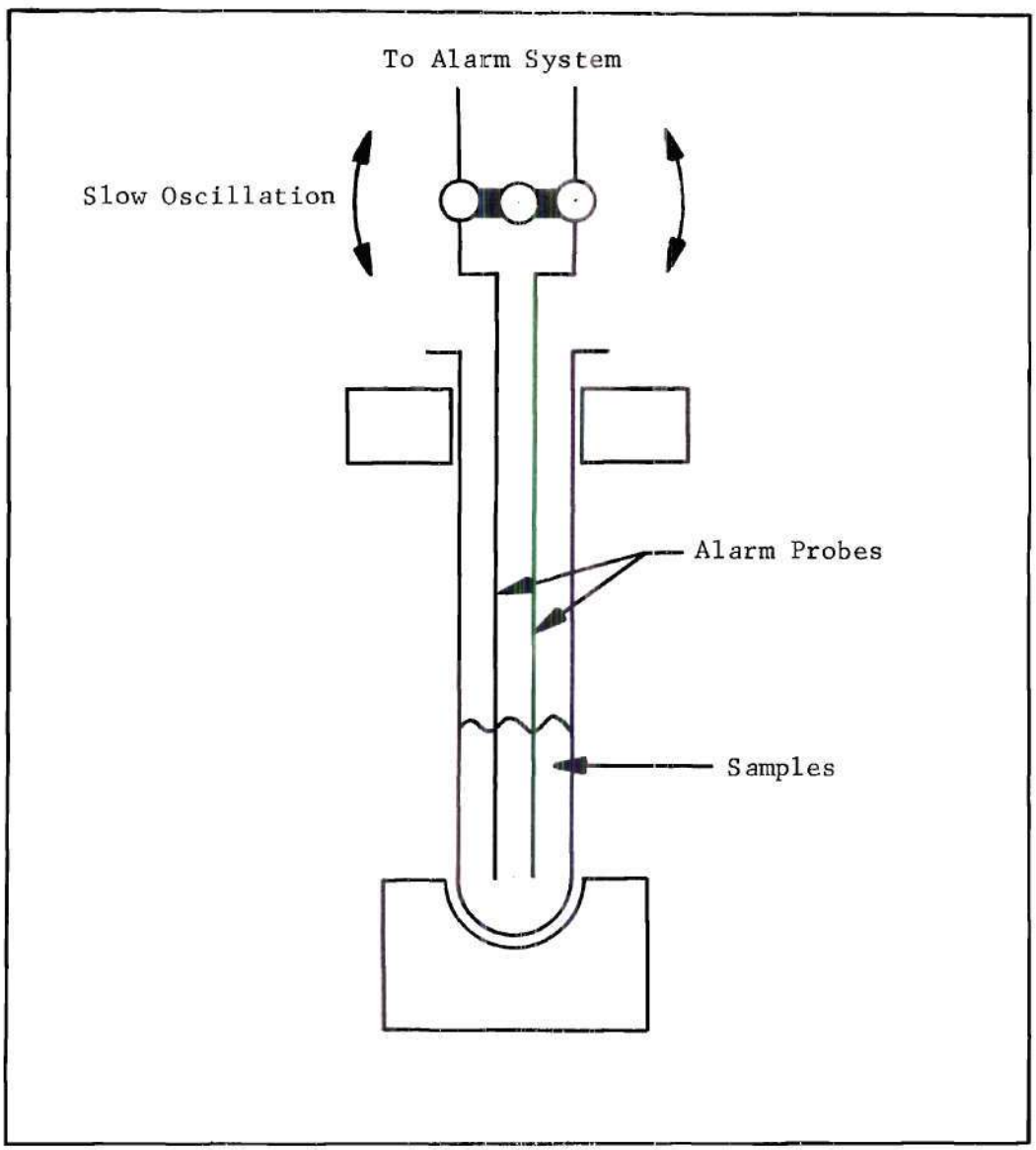


Figure 2. The Fibrometer.

the same consistency[13].

The thrombelastograph employs a relatively sophisticated method of measuring the viscosity of liquids. The principle of operation is described in Figure 3. In the unclotted state, the blood sample has a very small coefficient of viscosity, and the floating disc remains essentially stationary in the liquid blood as the cup oscillates. As the blood coagulates, the cup begins to drag the disc for small distances, causing the mirror to move. The mirror-disc combination tends to oscillate with increasing amplitude. A light beam impinges on the mirror and is reflected on to slowly-moving, light sensitive paper making a permanent record. The time interval between the initiation of the test and the point where the output amplitude settles out to a constant value is taken to be the clot time[14].

All three methods described depend on a change in the state of the sample from a liquid to solid during coagulation to determine the point at which blood clots. Physical agitation of the sample under test exists in all three testing procedures and causes the measured clot time to be less than its true value. Dr. Walter L. Bloom[15] has suggested that some electrical parameter in blood might be related to the clot time, and could be used to avoid the problem of agitation existing in the previously described methods. Variations in the electrical impedance of a blood sample undergoing coagulation might possibly be traceable to the clotting phenomenon. A useful electrical device for measuring impedance must be reliable, practical to implement, and economically feasible.

Feasibility of Clotting Time Determination Using Impedance Measurements

The use of direct current (d.c.) to measure the resistivity

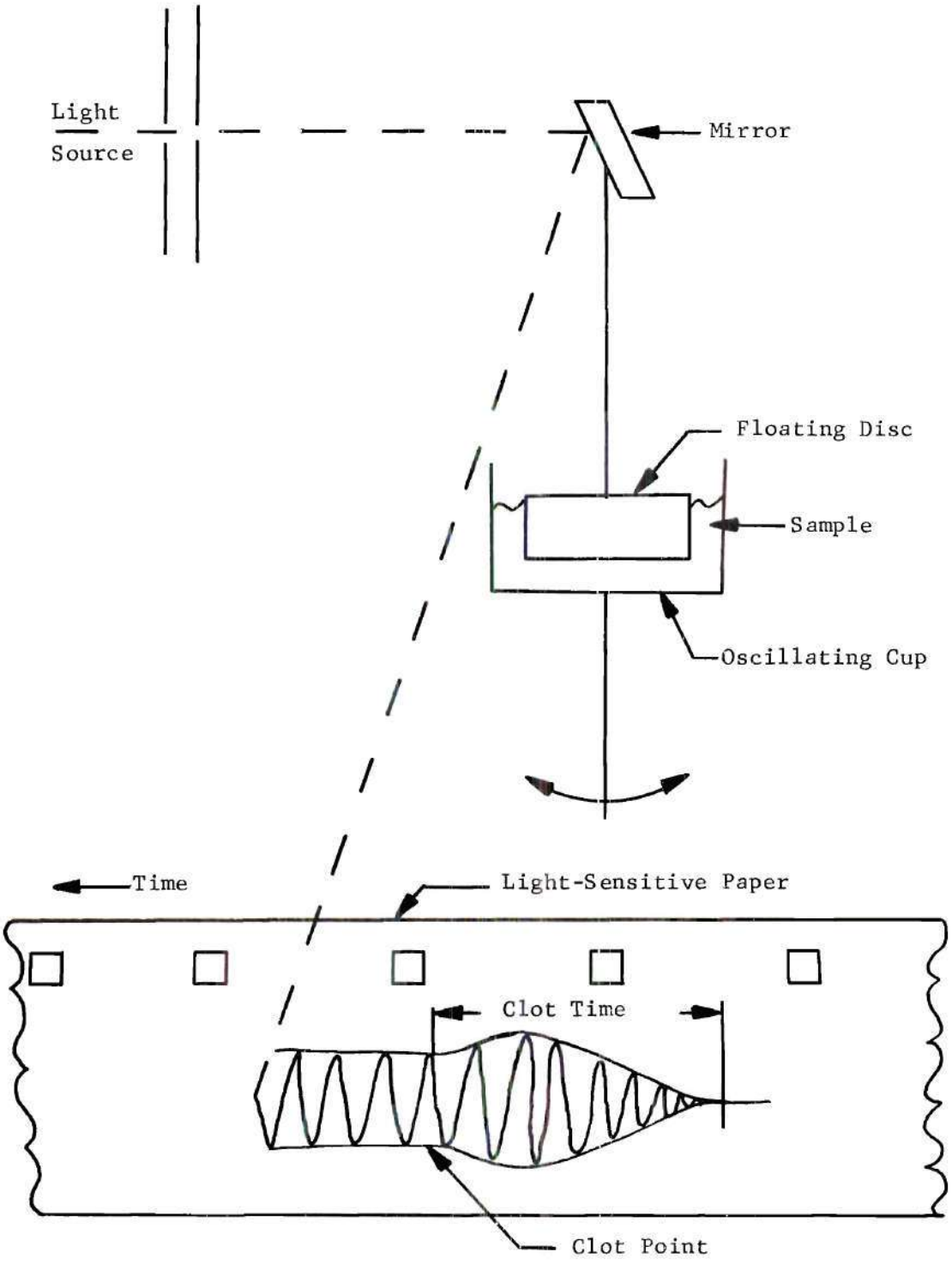


Figure 3. The Thrombelastograph.

component of blood impedance is known to cause electrolytic decomposition. Electrolysis of the ionized components in blood occurs at levels greater than two volts d.c.[16] Polarization of the electrodes causes an accumulation of hydrogen atoms on the positive electrode, so the resistivity of the blood sample depends somewhat upon the d.c. potential applied across the electrodes. Therefore the results obtained from this type of measurement are not accurate. For this reason, this approach was not taken.

By using a.c. it is possible to determine both the resistivity and permittivity of an electrolytic solution. Ferris[17] has used a balanced bridge technique to study the a.c. properties of generalized electrolytic solutions at a frequency of 1 kHz. This study has employed and adapted many of his ideas.

Gelatin as a Blood Substitute for Initial Impedance Measurements

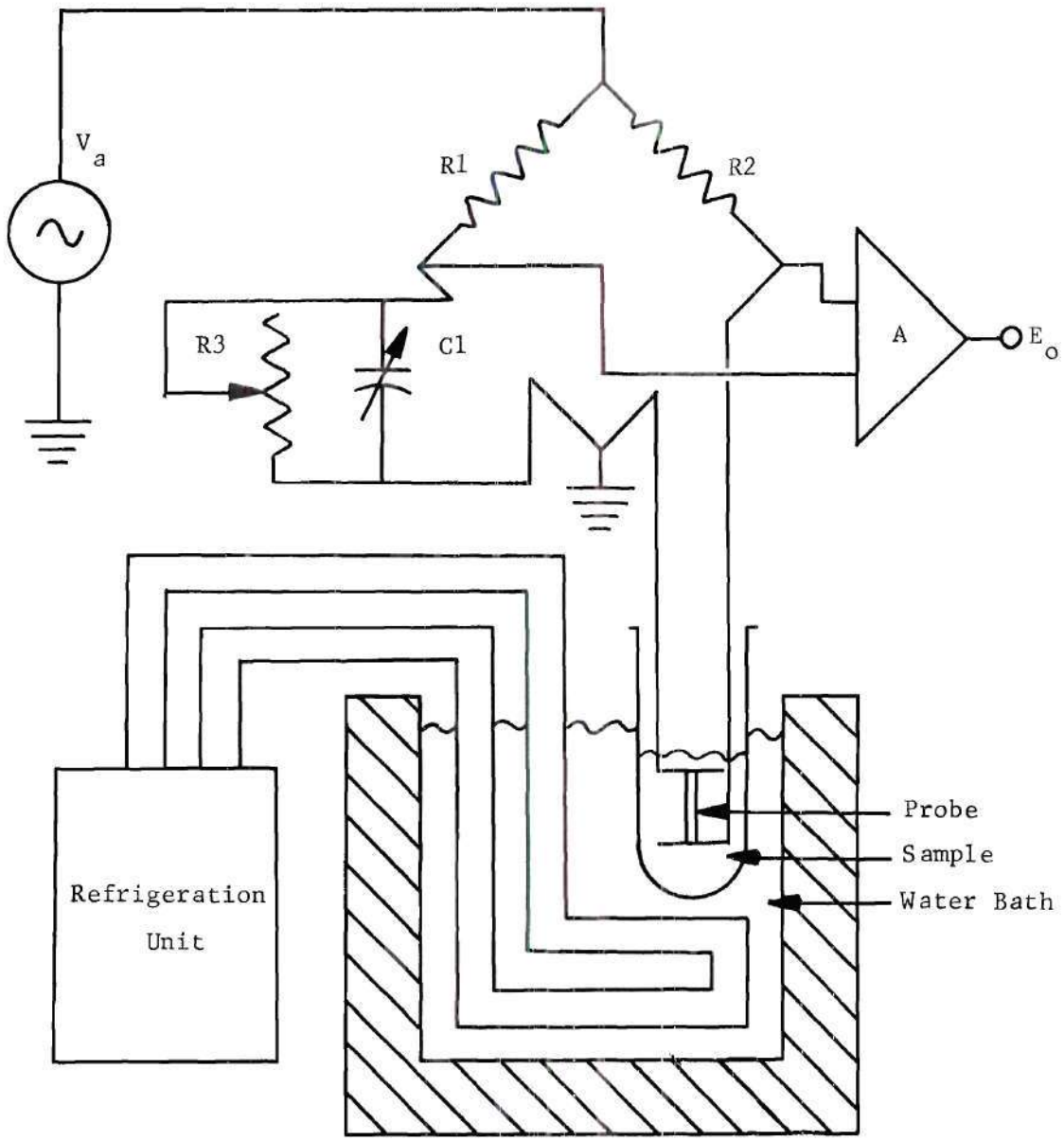
Whole human blood consists of red cells and plasma. The red cells carry oxygen and have no effect on the clot reaction. Thus the process of coagulation is often studied using only plasma[18]. Plasma contains water (91 per cent), protein (7 per cent), and a number of other more complex compounds (2 per cent)[19].

The clotting reaction of plasma is difficult to control. The reaction begins upon acquisition of the blood from the subject and continues until clot formation or until it is arrested by chemical means. This is usually accomplished by removing calcium ions as previously described (Chapter I, p.2). To develop a technique for studying blood, a blood substitute having similar physical and chemical properties was used.

The reaction in blood which finally forms the clot is the conversion of a protein in solution to a solid containing water and has been identified as the sol-gel reaction[20]. Since it was desired to study the electrical characteristics of plasma during this reaction, a solution having approximately the same concentration of protein and water as in plasma was chosen: 93 per cent water and 7 per cent gelatin (animal protein). By observing the electrical characteristics of this gelatin solution during gellation, it was possible to gain some insight into the type of results which might be expected from a similar test on blood.

A 7 per cent solution of gelatin in water is weak enough that it will not gel at body temperature. However, lowering the temperature below body temperature will cause the solution to gel. Thus the gellation process can be controlled by varying the solution's temperature.

Some work on the resistivity of gelatin solutions during gellation has already been done. Taft and Malm[21] have observed that the resistivity of gelatin solutions increases during gellation. Because this approach seemed promising, the setup shown in Figure 4 was used to test the 7 per cent gelatin solution. The gelatin was mixed with water heated to just below the boiling point. Five milliliters of the solutions were then poured into a test tube and a probe immersed into the sample. The test tube and probe were then placed in a temperature-controlled bath at 37° C. A refrigeration system was used to cool the bath gradually to a temperature of 25° C which was low enough to cause the solution to gel. Temperature was measured with a standard mercury thermometer. Readings of the equivalent resistance and capacitance necessary to balance the bridge shown in Figure 4 were taken in two degree increments between 37



V_a - audio oscillator, output 40 mV. P-P @ 1 kHz.
E_o - error voltage read on Tektronix type 535 Oscilloscope.
A - differential amplifier, gain = 80

R1 - R2-1.37 K, 1%
R3 - 2K potentiometer with vernier drive (10 turn)
C1 - capacitance decade box, (0.001 to 1.0 μfd)

Figure 4. Experimental Apparatus for Initial Tests Using Gelatin Solution.

and 25° C. Knowledge of the dimensions of the probe permitted calculation of the resistivity and permittivity of the solution. The results of the two test runs are shown in Figure 5.

The most interesting characteristic of the gelatin solutions studied is the marked change in slope of the resistivity curves. This change occurs immediately preceding the gel point. A similar effect was observed in other test runs. The slope changes by a factor of two or better, approaching nearly the horizontal.

Taft and Malm[22] suggest that the change in resistivity is tied to the availability of charge carriers. As a liquid, the gelatin solution uses both water molecules and gelatin molecules to transport charge. When the solution gels, however, the gelatin molecules become tied up in the amorphous solid structure of the gel and are then unavailable as charge carriers. This decrease in available carriers causes the observed increase in resistivity.

The vertical spacing between the two curves is attributed to small differences in the concentrations of the samples and its "ash content."[23] The purified gelatin used has a fairly high resistivity. The resistivity is sensitive to this "ash content" which is the presence of small impurities of highly ionizable compounds. Taft and Malm went to great lengths to free their samples of these impurities including several re-crystallizations and special procedures to avoid contamination in handling. Since the results of this test were to determine if such an approach with blood plasma seemed promising, elaborate purification procedures were not used.

Since this experimentation indicated that a weak gelatin solution

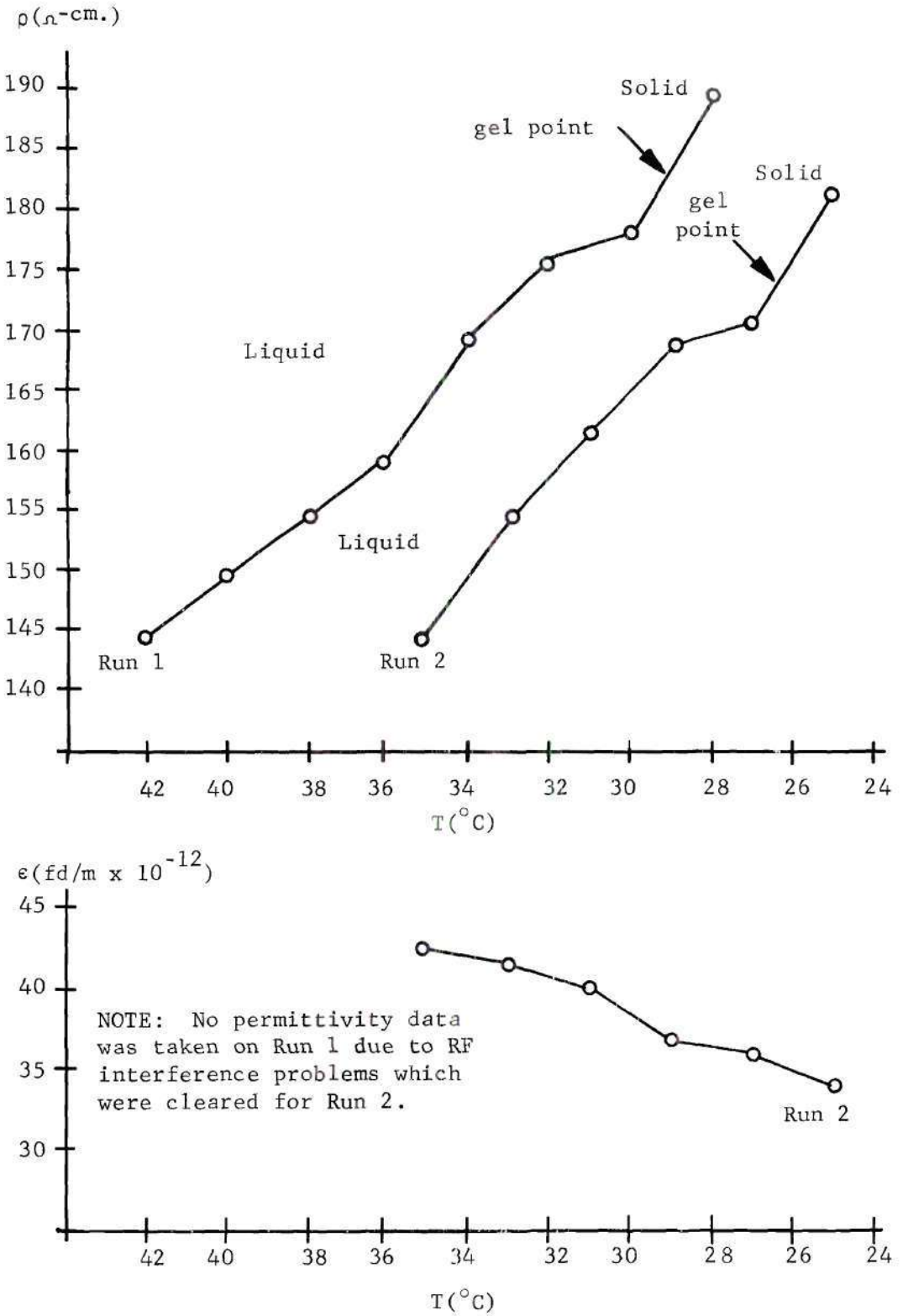


Figure 5. Initial Test Runs on Gelatin Solutions Showing Resistivity and Permittivity Variations with Temperature.

does change permittivity and resistivity during the sol-gel transformation, a similar study of the permittivity and resistivity of blood plasma during coagulation would be a worthwhile endeavor.

CHAPTER II

THESIS OBJECTIVE

The primary objective of this thesis was to determine if the resistivity curve of human blood plasma displays a similar change in slope immediately before the final stage of the clot reaction as the gelatin sample. This change could possibly be used to identify electrically the clot point, thereby avoiding the problem of physical agitation associated with the methods described in Chapter I.

A refined version of the balanced bridge system used in the initial measurements will be employed to determine the resistivity of plasma during coagulation. The bridge system will be redesigned in order to provide resistivity measurement accuracy of less than 0.3 per cent. Data will also be taken to provide at least qualitative information concerning the permittivity characteristics of human blood plasma during coagulation. Approximately ten samples will be tested to insure the repeatability of the experiment.

A secondary objective of the thesis is to correlate the resistivity variations of the blood sample with the clot time, as determined by an independent method. A Lee-White test will be used to measure the clot time. A mathematical expression will be fitted to the experimental resistivity data obtained. Knowledge of an equation relating resistivity to the elapsed time from the initiation of the clot reaction might allow the prediction of the clot times of other samples. This would be accomplished by taking several measurements before coagulation and

extrapolating the resistivity curve to the clot point.

From the experience gained working with human blood and from the experimental data obtained, it may be possible to design a practical device to determine the clot time of plasma samples. Such a device could be of great value in a medical laboratory from the standpoints of accuracy and efficiency.

CHAPTER III

THE MEASUREMENT SYSTEM COMPONENTS

General Considerations

One of the basic problems in designing a system to monitor a biological phenomenon is the alteration of the parameters of interest of the system under test by the measurement equipment. As previously mentioned, the primary problem in making electrical impedance measurements on blood or blood plasma is electrolytic decomposition. A previous study by Lamb[22] indicates that electrolysis of a blood sample was not observed until the input power exceeded approximately 20 mW.

Figure 6 shows the system used to measure the resistivity and permittivity of blood plasma. A low level input signal from the generator is employed to avoid the electrolytic decomposition problem. By limiting the input voltage of the source, V_s', to 100 mV. peak to peak, the voltage swing across the sample under test cannot exceed this level. Preliminary impedance measurements made on a representative collection of plasma samples are shown in Table 1. These measurements indicate that the equivalent impedance always had a resistive component which exceeded 50 ohms. The probe shown in Figure 7 was employed for these preliminary measurements. Therefore, the maximum power which could be dissipated in the sample is:

$$P = V^2/R = \frac{\left[(100/2\sqrt{2}) \times 10^{-3}\right]^2}{50} = 25 \times 10^{-6} \text{ W.}$$

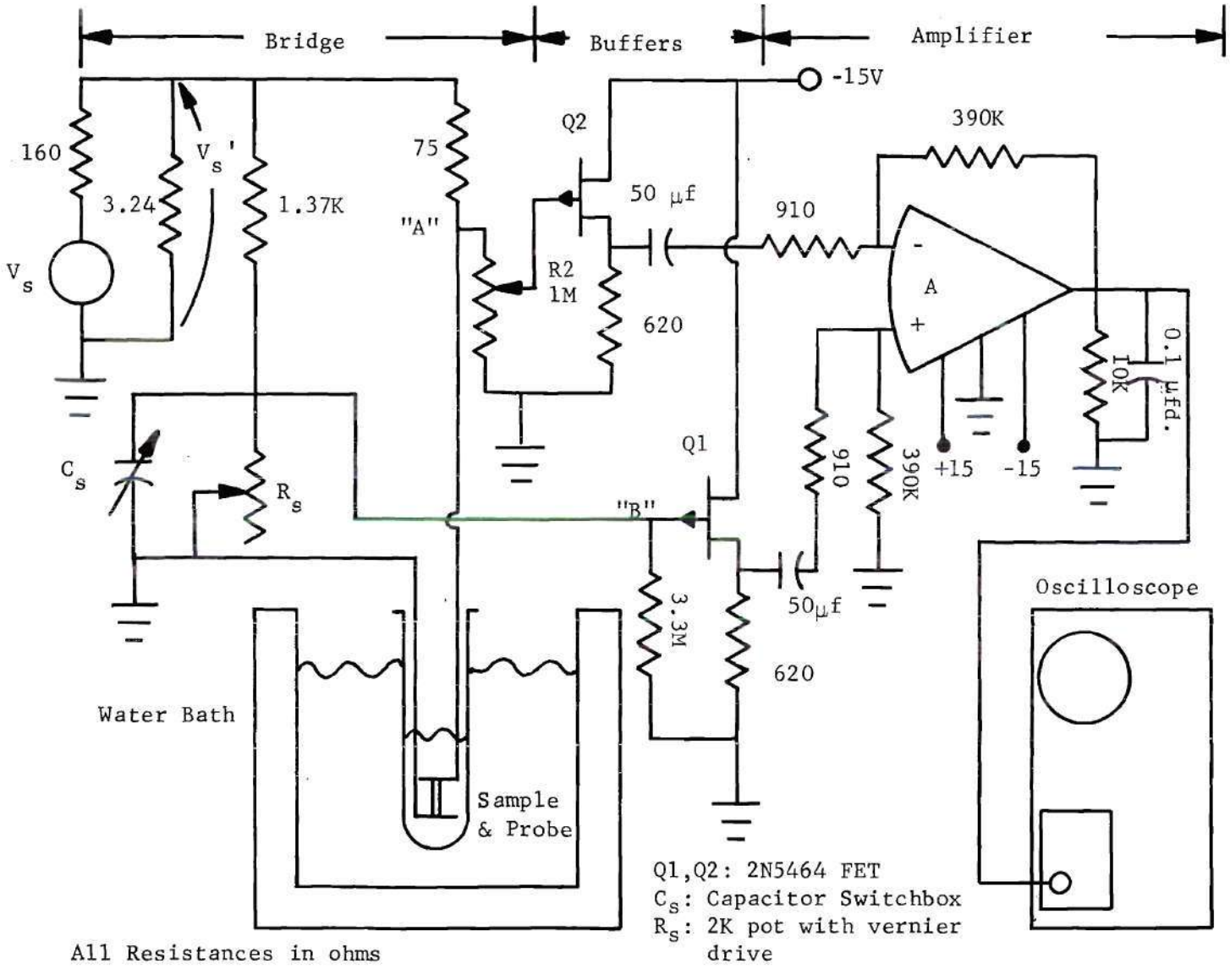
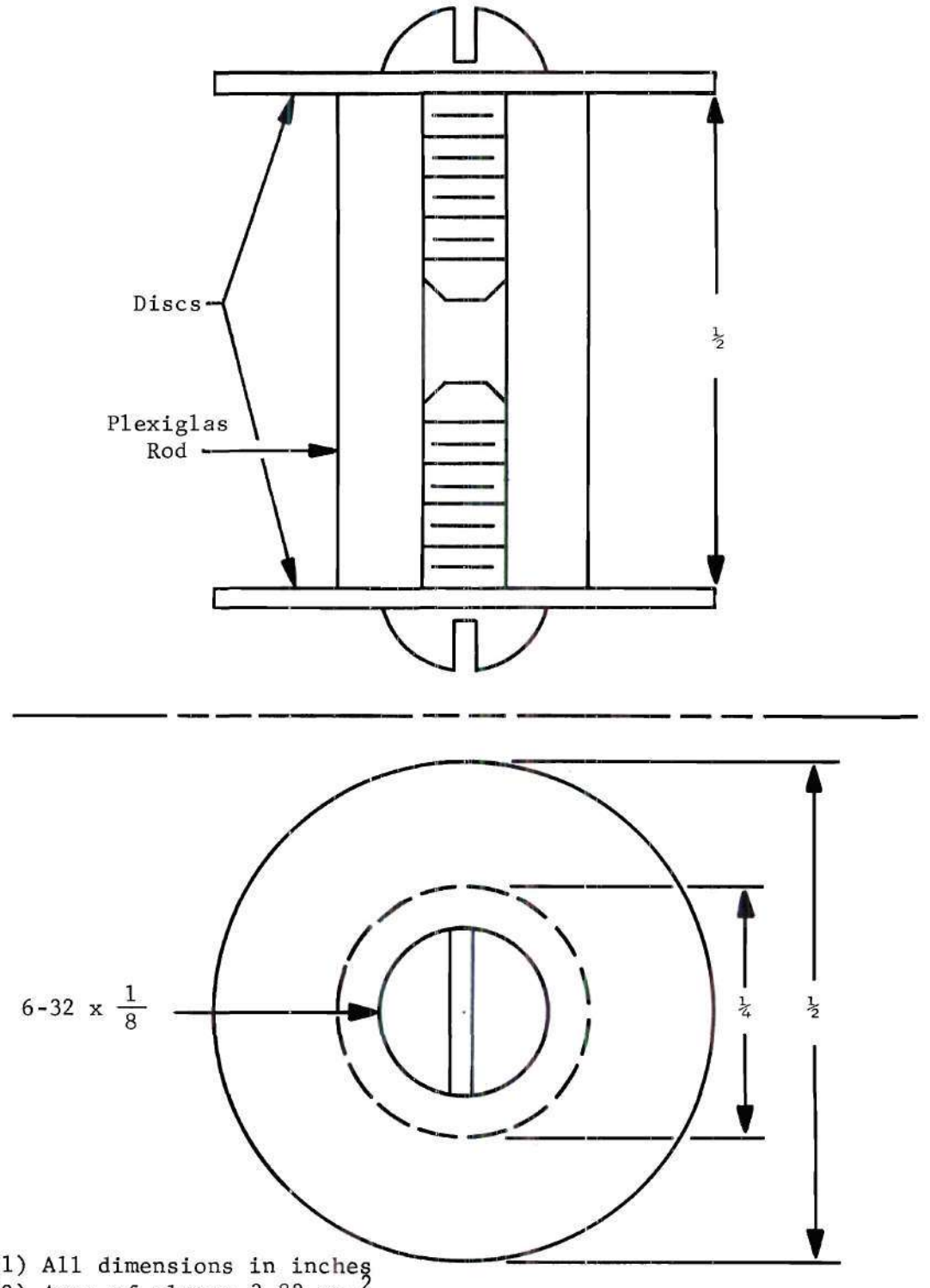


Figure 6. The Bridge Measurement System.

Table 1. Preliminary Test Data Showing Impedance Variations In Human Blood Plasma

T	R	C
1.0	88.4	0.40
1.5	86.6	0.41
2.0	86.0	0.41
2.5	86.0	0.41
3.0	86.0	0.42
*3.5	85.8	0.42
1.0	95.4	0.40
1.5	92.1	0.40
2.0	91.5	0.41
2.5	90.5	0.41
3.0	89.9	0.41
3.5	89.5	0.41
*4.0	89.5	0.41
4.5	89.5	0.41
1.0	79.3	0.51
1.5	78.4	0.52
2.0	78.0	0.52
2.5	78.0	0.52
*3.0	78.0	0.52

T time in minutes from recalcification of blood plasma
R resistance of recalcified plasma sample in ohms
C capacitance recalcified plasma samples in microfarads
* indicates time when plasma sample was observed to be clotted



(1) All dimensions in inches
(2) Area of plates 3.82 cm.^{2}

Figure 7. The Probe.

This maximum power is three orders of magnitude less than the 20 mW at which electrolysis was observed. Therefore, it is assumed that no significant electrolysis occurs.

The Probe

The probe has been shown in Figure 7. It is basically a parallel-plate capacitor made of two ½-inch tinned sheet metal discs separated by a plexiglas rod. By using the fundamental definitions of resistance and capacitance for a straight rectangular or cylindrical length of material, the resistivity and permittivity can be expressed as:

$$\rho = RA/L \tag{4}$$

$$\epsilon = CL/A \tag{5}$$

where

ρ = resistivity of the dielectric medium between the plates

ϵ = permittivity of the dielectric medium between the plates

R = equivalent resistance of the sample in ohms

C = equivalent capacitance of the sample in μfd

L = separation distance of the plates in centimeters

A = surface area of the plates in centimeters squared

Inserting the dimensions from Figure 7 into equations (4) and (5),

$$\rho = 2.96\ R \text{ ohm-centimeters} \tag{6}$$

$$\epsilon = 33.85\ C \text{ farads/meter.} \tag{7}$$

These equations can be used to closely approximate the actual resistivity and permittivity of the sample. Since the diameter of the probe contacts is almost 13 mm, and the inside diameter of the test tubes used for these

tests is nominally 15 mm, there is essentially a straight column of plasma between the contact plates. Thus the probe arrangement is similar to setups used to measure the resistivity and permittivity of standard lengths of material. A more complicated analysis accounting for fringing effects of the fields between the plates was deemed unnecessary since this work is not intended to present standard values for ρ and ϵ but rather to determine changes which occur in these parameters during the clotting process.

The probe itself has some intrinsic capacitance and resistance. The probe parameters were measured on a Hewlett-Packard model 4260A Universal Impedance Bridge. It was found that the probe capacitance in air was 47 pf. The probe resistance exceeded one megohm.

From the preliminary data (Table 1) the minimum value of C encountered was 0.40 μfd. Therefore, the shunt capacitance is almost four orders of magnitude less than the intrinsic probe capacitance and can be neglected. Similarly, the maximum equivalent resistance encountered from the preliminary was 149 ohms, which is nearly four orders of magnitude less than the intrinsic probe resistance. Therefore, the one megohm shunt resistance is also neglected.

Support Equipment for the Measuring System

A water bath was used to control the temperature of the sample. The bath employed was large enough to maintain the sample at a constant temperature. The unit used in this experiment was a Precision Scientific bath having an internal heater controlled by a thermostat. Its water capacity is roughly twelve quarts. Since the clot time of blood is strongly influenced by its temperature, it is necessary that the

temperature shift be no greater than $\pm\frac{1}{2}^{\circ}$ from the 37° C center value[23]. This requirement was easily met with the bath used.

A Tektronix type 585 oscilloscope with a #82 plug-in was used to read the output voltage of the detector. Since the noise pickup throughout the system causes an output noise level of roughly five to ten millivolts peak, a vertical sensitivity of 50 mV/cm was chosen as the optimum setting for the oscilloscope.

The temperature standard was a centigrade mercury thermometer. It was suspended such that it was immersed in the bath between the two test tubes containing the blood plasma samples.

Adjustment and Calibration

The two field effect transistors used in the buffer stages (Figure 6) were not matched units. Potentiometer R_2 was inserted to balance out small differences in the characteristics of the transistors, assuring that equal inputs to the buffer stages cause a minimum output at the detector. To set R_2 the inputs at "A" and "B" are shorted together, the input voltage to the bridge, V_s', is set at 100 mV and R_2 is adjusted for minimum output at the detector.

CHAPTER IV

EXPERIMENTAL RESULTS

Procedure

Before measurement of the blood samples' resistance and capacitance could begin, it was necessary to allow the temperatures of the test equipment, water bath, and blood samples to stabilize. The thermostat controlling the temperature of the water bath was set to 37° C. Testing was not begun until the temperature of the water bath had remained constant for at least 30 minutes. All test equipment was turned on at least 30 minutes prior to the initiation of measurements and checked for proper settings. A resistor and capacitor whose values were known by checking on the Hewlett-Packard 4260A bridge were placed across the unknown bridge leg of Figure 6 to check system calibration. The blood samples were allowed to stand for approximately twelve hours, or until the plasma separated from the red cells.

A 0.02 molar solution of calcium chloride was used to recalcify the plasma samples and initiate coagulation[24]. The solution was warmed in the bath to 37° C before it was used.

The test procedure is outlined in Figure 8. Whole blood samples from the medical laboratory of Piedmont Hospital in Atlanta were used in this test. These samples were treated at the time of acquisition with the compound EDTA[25] to prevent coagulation. These samples are normally used for blood count determinations. The process by which EDTA prevents coagulation is not completely understood. However, it is known

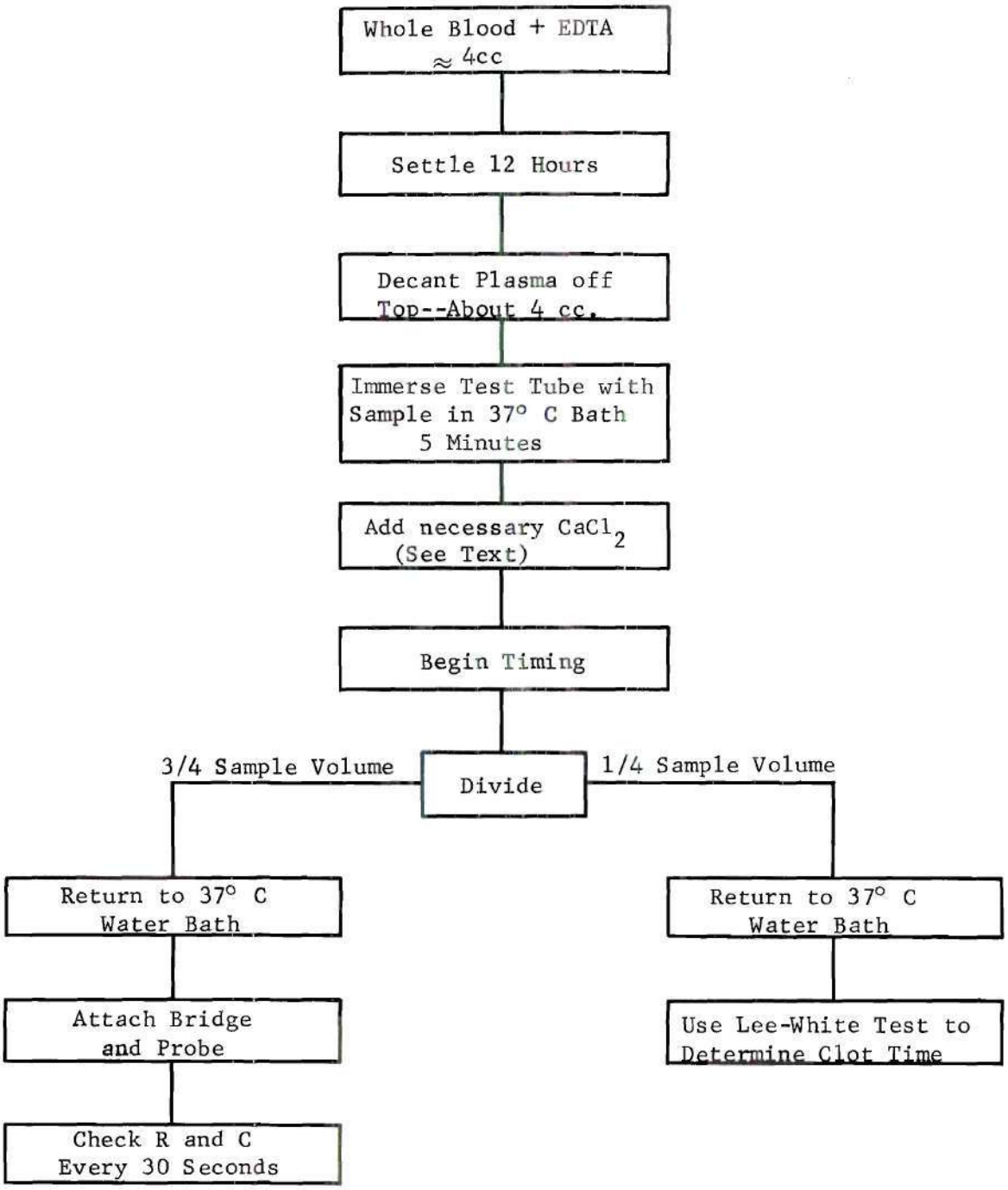


Figure 8. The Test Procedure.

that its addition to the blood alters the true clotting time[26]. Nevertheless, the sample can be made to clot if calcium ions in the form of a calcium chloride solution are added. Therefore, while the clot time observed is not the true clot time, it was possible to determine a clot time by performing the Lee-White test simultaneously with the resistivity and permittivity measurements.

Because the resistivity and permittivity of plasma are known to be sensitive to temperature, the sample must be warmed to the temperature of the bath. Observation has shown that approximately five minutes were required for a four to six cubic centimeter sample initially at room temperature to reach the 37° C temperature of the bath.

The clot time of EDTA treated samples when recalcified depends on two major factors, the quantity of $CaCl_2$ added, and the age (or length of time since acquisition) of the sample. Because the exact time elapsed since acquisition was generally not known, it was necessary to make a test run with each batch of samples to determine the proper amount of calcium chloride to add for a three to five minute clotting time. Initially, a ratio of four parts plasma to one part 0.02 molar $CaCl_2$ was used. This ratio was readjusted until the clotting time of the test sample fell within the desired limits.

The Lee-White testing procedure has several variations, and the particular method used in this research was a compromise between simplicity and accuracy. The use of three samples for each test run would have required about six milliliters of plasma, which is approximately twice the amount available from Piedmont Hospital. A Lee-White test performed on a single sample of one milliliter of blood plasma provides a first

order approximation to the clot time if the sample is not agitated excessively. This testing procedure of timing a single sample for coagulation with a wristwatch results in a determination of the plasma clotting time. To establish a reference consistency, the sample was considered clotted when it coagulated to a consistency such that it adhered to the side of the test tube when the tube was turned to a horizontal position.

After some practice, the bridge could be balanced and the second sample checked for coagulation with the Lee-White test described in about ten seconds. Measurements on both samples were begun 25 seconds after the addition of the $CaCl_2$ solution to the plasma samples and were usually finished within ten seconds. The remaining time between measurements was used to record the data manually. This procedure was repeated at thirty second intervals until the sample was observed to be clotted. The total time involved in testing each sample of plasma was approximately fifteen to twenty minutes.

Data

The reduction of the raw data into values of ρ and ϵ requires the computation of two conversion constants. To convert scale values on the resistance and capacitance standards to resistivity and permittivity involves three steps.

First the scale value is converted to resistance in ohms and capacitance in microfarads by multiplying the scale reading value by the appropriate conversion factor. The full resistance of the potentiometer used for the resistance standard is 2000 ohms; therefore, the resistance required for balance of the bridge, R_s, is

$$R_s = 2000\ F_s \tag{8}$$

where F_s is the scale reading on the vernier whose full scale value is 1.000. The capacitance required for balance, C_s, is read directly in microfarads from the switch scales on the capacitance decade boxes used.

The second step involves use of the bridge equation (4a) derived in Appendix A,

$$Z_o = \frac{Z_2}{Z_1}\ Z_s \tag{9}$$

to derive expressions for R_o and C_o, the equivalent resistance and capacitance of the plasma sample and probe which comprise the unknown leg of the bridge.

$$Z_o = \frac{Z_2}{Z_1}\ Z_s \tag{10}$$

or

$$\frac{1}{Y_o} = \frac{Z_2}{Z_1}\ \frac{1}{Y_s} \tag{11}$$

Z_2/Z_1 is a constant ratio of two resistances, therefore, (11) becomes

$$\frac{1}{G_o + j\ B_o} = \frac{K}{G_s + j\ B_s} \tag{12}$$

where

$$K = \frac{Z_2}{Z_1} \tag{13}$$

Rearranging (12) gives

$$G_s + j\,B_s = K\,(G_o + j\,B_o) \tag{14}$$

equating real and imaginary parts of (13),

$$B_o = \frac{B_s}{K} \tag{15}$$

$$G_o = \frac{G_s}{K} \tag{16}$$

since

$$B_o = \omega C_o \tag{17}$$

$$B_s = \omega C_s \tag{18}$$

$$G_o = 1/R_o \tag{19}$$

$$G_s = 1/R_s \tag{20}$$

equations (15) and (16) become

$$R_o = K\,R_s \tag{21}$$

$$C_o = C_s/K \tag{22}$$

Using the component values shown in Figure 6, page 17 to compute K,

$$R_o = 0.087\ (2000)\ F_s \tag{23}$$

$$C_o = 11.42\ C_s \tag{24}$$

Finally, using equations (6) and (7) from Chapter III to convert R_o and

C_o to equivalent values of ρ and ϵ

$$\rho = 2.96\ R_o = 5.19\ F_s \quad \text{ohm-cm.} \tag{25}$$

$$\epsilon = 33.85\ C_o = 386\ C_s \quad \text{farads/meter} \tag{26}$$

the raw and reduced data is summarized in Table 2.

Analysis of Results

The resistivity of all the plasma samples tested decreased during coagulation. Nine of the ten samples' resistivity decreased at least 4 per cent from the initial value of resistivity measured. The one exception was Run #9 which decreased only 2.5 per cent. Since the maximum theoretical error that can exist in the bridge is 0.3 per cent, these changes are more than an order of magnitude above the maximum theoretical error present in the bridge system.

Figure 9 is a plot of the data from Run #1, and is used to illustrate the method employed to correlate the experimental impedance data with the clot time determinations. The plot suggests an exponential curve for the plasma's resistivity as a function of time. To check this hypothesis, calculations were performed using the experimental data to determine the time constant of the exponential function.

An exponential function of time can be expressed as:

$$\rho(\tau) = P_f + (P_i - P_f)\ e^{-k\tau} \tag{27}$$

where

P_f = final value of the resistivity

Table 2. Experimental Data on the Impedance Variations of Blood Plasma During Coagulation

	T	R_o	ρ	C_o	$\epsilon \times 10^{-12}$
Run #1					
	1.5	96.4	286	0.37	12.4
	2.4	94.8	281	0.37	12.4
	2.5	93.8	278	0.37	12.4
	3.0	93.1	276	0.37	12.4
	3.5	92.7	275	0.37	12.4
	4.0	92.7	275	0.37	12.4
	4.5	92.4	274	0.37	12.4
	*5.0	92.4	274	0.37	12.4
Run #2					
	1.0	101	298	0.43	14.7
	1.5	96.1	285	0.46	15.5
	2.0	95.2	282	0.46	15.5
	2.5	94.2	279	0.46	15.5
	3.0	94.2	279	0.46	15.5
	*3.5	94.2	279	0.46	15.5
Run #3					
	1.0	98.5	292	0.43	14.7
	1.5	95.5	283	0.43	14.7
	2.0	93.5	277	0.43	14.7
	2.5	92.1	273	0.43	14.7
	*3.0	91.5	271	0.43	14.7
	3.5	91.1	270	0.43	14.7
	4.0	90.9	269	0.43	14.7
Run #4					
	0.5	91.8	272	0.46	15.5
	1.0	89.5	265	0.46	15.5
	1.5	87.5	259	0.46	15.5
	2.0	86.5	256	0.46	15.5
	2.5	85.4	253	0.46	15.5
	3.0	84.7	251	0.46	15.5
	3.5	84.4	250	0.46	15.5
	4.0	84.4	250	0.46	15.5
	4.5	84.1	249	0.46	15.5
	*5.0	84.1	249	0.46	15.5

* Indicates Time When Plasma Sample Was Observed To Be Clotted

Table 2. Experimental Data on the Impedance Variations of Blood Plasma During Coagulation (Continued)

	T	R_o	ρ	C_o	$\varepsilon \times 10^{-12}$
Run #5					
	1.0	101	298	0.42	14.4
	1.5	97.2	288	0.43	14.7
	2.0	96.1	285	0.43	14.7
	2.5	95.9	284	0.43	14.7
	3.0	95.9	284	0.45	15.1
	3.5	95.5	283	0.45	15.1
	4.0	95.5	283	0.45	15.1
	4.5	95.2	282	0.45	15.1
	*5.0	94.8	281	0.45	15.1
Run #6					
	0.5	104	308	0.42	14.4
	1.0	101	298	0.42	14.4
	1.5	98.5	292	0.41	14.0
	2.0	98.2	291	0.41	14.0
	2.5	97.5	289	0.41	14.0
	*3.0	97.5	289	0.41	14.0
	3.5	97.5	289	0.41	14.0
	4.0	96.9	287	0.41	14.0
	4.5	96.4	286	0.41	14.0
Run #7					
	1.0	130	384	0.37	12.4
	1.5	126	373	0.35	12.0
	2.0	125	363	0.35	12.0
	2.5	123	364	0.35	12.0
	3.0	122	362	0.35	12.0
	3.5	121	360	0.35	12.0
	4.0	121	359	0.35	12.0
	4.5	120	358	0.35	12.0
	*5.0	120	358	0.35	12.0
Run #8					
	1.0	135	400	0.40	13.4
	1.5	133	389	0.40	13.4
	2.0	129	382	0.40	13.4
	2.5	128	378	0.40	13.4
	3.0	127	377	0.40	13.4
	*3.5	127	376	0.40	13.4

* Indicates Time When Plasma Sample Was Observed To Be Clotted

Table 2. Experimental Data on the Impedance Variations of Blood Plasma During Coagulation (Concluded)

	T	R_o	ρ	C_o	$\epsilon \times 10^{-12}$
Run #9					
	1.0	149	441	0.30	10.1
	1.5	147	435	0.30	10.1
	2.0	146	431	0.30	10.1
	2.5	145	428	0.30	10.1
	3.0	144	427	0.30	10.1
	3.5	144	427	0.30	10.1
	*4.0	144	426	0.30	10.1
Run #10					
	1.0	132	389	0.51	17.4
	1.5	128	378	0.51	17.4
	2.0	126	374	0.51	17.4
	2.5	125	371	0.51	17.4
	3.0	125	369	0.51	17.4
	3.5	124	368	0.51	17.4
	4.0	124	368	0.51	17.4

* Indicates Time When Plasma Sample Was Observed To Be Clotted

T in minutes

R_o in ohms

ρ in Ω-cm.

C_o in μfd.

ε in farads/meter

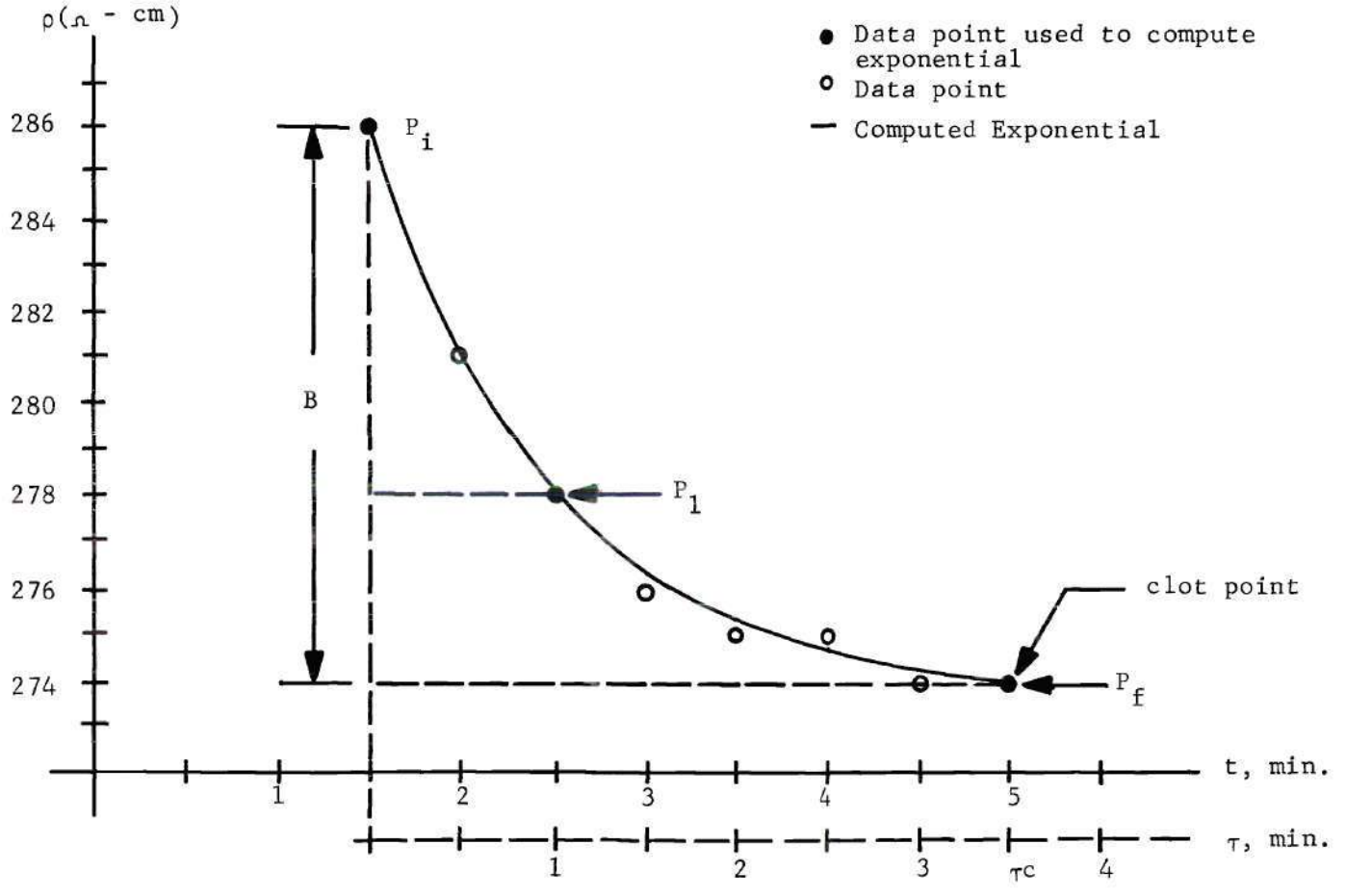


Figure 9. Data from Run #1.

P_i = initial value of the resistivity

$k = 1/\alpha$ = argument of the exponential

α = time constant of the exponential

Since the value at t = 0 of the resistivity was not measured, the time axis is shifted to the right so that the first data point is:

$$\tau = t - t_o \tag{28}$$

where t_o is the time at which the first data point was taken.

Determination of P_f, P_i, and k is straightforward. P_f and P_i are taken directly from the graph as shown in Figure 9. The argument, k, is computed by choosing a point τ = 1 sec and obtaining the resistivity for that point from the data. If ρ (1) = P_1 then

$$P_1 = P_f + (P_i - P_f)\, e^{-k(1)} \quad . \tag{29}$$

Rearranging, equation (29) becomes

$$e^k = \frac{P_i - P_f}{P_1 - P_f} \tag{30}$$

Taking the natural logarithm of both sides of equation (30) gives k as

$$k = \ln \left[\frac{P_i - P_f}{P_1 - P_f} \right] \tag{31}$$

Using these constants, the exponential curve is drawn on the graph (Figure 9) for comparison. As shown in Figure 9, a true exponential computed on the basis of three data points along the curve closely approximates the shape of the data curve. All ten of the test runs were

studied using this technique. With the exception of Run #5 which had one point 0.5 per cent off of the value computed for the exponential, all the data on the ten runs matched the computed exponential within the 0.3 per cent error range of the measurement system derived in Appendix A.

A comparison between the observed clot time and the time constant of the exponential equation for each test run is given in Table 3(a) and summarized by clot time in Table 3(b). The results of this comparison are inconclusive. There appears to be no simple relationship between the clot time, τ_c, and the time constant, α, of the corresponding exponential equation. It is possible that this is due to error in the measurement of the clot time using the simplified Lee-White test. Since the plasma was checked at thirty second intervals, it is possible that each clot time may be up to thirty seconds less than the clot time recorded. And as was mentioned in Chapter I, there is inevitably some operator judgement error involved in determining the clot time by the Lee-White test.

The data does fit very closely the shape of the exponential. However it cannot be said that the curve is exponential, since such a statement would require a complicated mathematical analysis to prove that no curve exists which better fits the data points. It can be said that a theoretical exponential function can be fitted to the data to an accuracy greater than 0.3 per cent, and an exponential variation with time can be assumed to be a valid expression within the limitations of the measurement system. It is also observed from the data that the clot time always occurs near the point where the tangent to the curve approaches the

Table 3. Observed Clot Times and Time Constants of Exponentials

Run	T_c	τ_c	k	α
1	5.0	3.5	1.10	0.91
2	3.5	2.5	1.84	0.54
3	3.0	2.5	1.06	0.94
4	5.0	4.5	0.88	1.13
5	5.0	4.0	1.67	0.60
6	3.0	2.5	1.85	0.54
7	5.0	4.0	0.86	1.16
8	3.5	2.5	1.23	0.82
9	4.0	3.0	0.79	1.27
10	4.0	3.0	0.80	1.25

(a)

Observed τ_c	Corresponding α's
2.5	0.54, 0.94, 0.54, 0.82
3.0	1.27, 1.25
3.5	0.91
4.0	1.13, 0.60, 1.16

(b)

T_c - measured clot time

τ_c - clot time transferred to τ axis (see Figure 9)

k - argument of exponential

α - 1/k, time constant of exponential

horizontal. This observation must also be judged considering the fact that the observed clot time may be as much as thirty seconds in error.

As is shown in Appendix A, the larger theoretical error of 2.2 per cent associated with the reactive component measurement prevents more than a qualitative conclusion concerning its changes during the coagulation process. As can be seen in Table 2, the permittivity never changed more than 1 per cent from its initial value at the beginning of the test run. Since the error associated with the permittivity measurement is twice this amount, it can be said that the permittivity remains relatively constant throughout the coagulation process. However, this result may be due to a lack of sensitivity in the measurement system for detecting reactive variations in the sample of the same magnitude as for the resistivity variations. The experimental data does indicate that no large reactive variation greater than 2.2 per cent was evident during the test runs.

CHAPTER V

CONCLUSIONS

The resistivity of human blood plasma decreases during coagulation. Since the resistivity of the gelatin sample solutions used for initial studies increased, it can be concluded that gelatin solutions do not accurately simulate resistivity characteristics of human blood plasma during coagulation after recalcification, when the gellation is forced by a temperature change.

The decrease of the resistivity of the plasma during coagulation is significant and always exceeded four per cent of the initial value measured. This change is not attributable to inaccuracies in the bridge, since the bridge accuracy is an order of magnitude better than the four per cent resistivity changes during coagulation.

Changes in the reactive component of the plasma's impedance (and consequently the measurement of the permittivity) are less well defined than the resistive component primarily due to the significant error (2.2 per cent) associated with this measurement. However it can be said that no change in the permittivity greater than 2.2 per cent was observed.

The resistivity data of the plasma sample can be fitted to an exponential curve within the limits of accuracy of the bridge system. The point where changes in the resistivity become less than the 0.3 per cent error range of the bridge can be used to determine the clot point.

There appears to be some correlation between the clot time as determined by the Lee-White test and the time constant of the exponential

curve associated with each test run. However the experimental procedure in attempting to adjust the clot time to between three and five minutes tends to minimize the spread of the time constants and consequently minimizes any difference between clot times and time constants. The accuracy of the correlation is also impaired by the thirty second uncertainty associated with measurement of the clot time by the Lee-White test. In the case of a 3.5 minute clot time, the true clot time may be as much as 15 per cent less than the 3.5 minutes observed. Additional work must be done to establish a definite correlation between the time constant and the observed clot time.

Time did not permit the application of these data to the development of a device to measure the clotting time by electrical means, but from the study, such a device seems practical.

CHAPTER VI

SUGGESTIONS FOR FURTHER STUDY

The results of this work suggest many areas of further study. Four of the most promising of these are discussed below.

1. The literature search did not discover any studies on the changes in blood permittivity during coagulation. Original research dealing with permittivity might reveal significant variations tracable to the chemical changes of blood during coagulation. However, any experimental research must utilize a bridge with greater sensitivity than that employed in this research.

2. A continuous method of resistivity measurement could eliminate the inaccuracy of timing associated with this work. The times indicated in this work are accurate to only ± five seconds, since the total time required to measure the resistivity and permittivity of the sample was approximately ten seconds. An exact curve on the time axis could further clear the question of whether or not the resistivity as a function of time is exponential.

3. From a continuous data curve, it may be possible to prove mathematically that the curve is best fitted by an exponential. Such a proof would allow prediction of the clot time before coagulation occurs.

4. If it can be proved that the resistivity of blood plasma during coagulation is exponential, the clot time of a sample could be predicted by taking three (or more) measurements of the resistivity before coagulation, by computing the time constant of the exponential and

determining by experiment the relationship between the exponential time constant and the clotting time.

5. Physicians use many different procedures to measure the clotting characteristics of blood. Before information on the electrical characteristics of blood during coagulation can be of value, it must be correlated with existing measurement methods, perhaps by comparing clot times determined with the electrical procedure to times determined by a presently used method, or by using standard solutions which clot at a previously determined point as measured by a commonly used procedure such as thrombelastographic study.

APPENDIX A

ANALYSIS OF THE BRIDGE SYSTEM ERRORS

The analysis of the measurement system to determine bridge accuracy involves two sources of error:

1. Bridge Sensitivity - a measure of how large a deviation from the null condition can remain undetected at the output of the detector. This error depends upon the type of bridge employed, the output impedance of the signal source, and the input impedance of the detector.
2. Readout errors - readability of the scale graduations on the measurement standards.

Sensitivity

The sensitivity of a bridge is the unbalance voltage across the detector per unit generator voltage produced by a small fractional change in the impedance of a bridge arm measured from the impedance value for exact balance. Mathematically,

$$S = \frac{\Delta E_d \ / \ E}{\Delta Z \ / \ Z} \tag{1a}$$

A generalized bridge is shown in Figure A1. If I_d, the detector current is zero, the bridge is balanced[27]. Under this condition,

$$V_1 = V_2 \tag{2a}$$

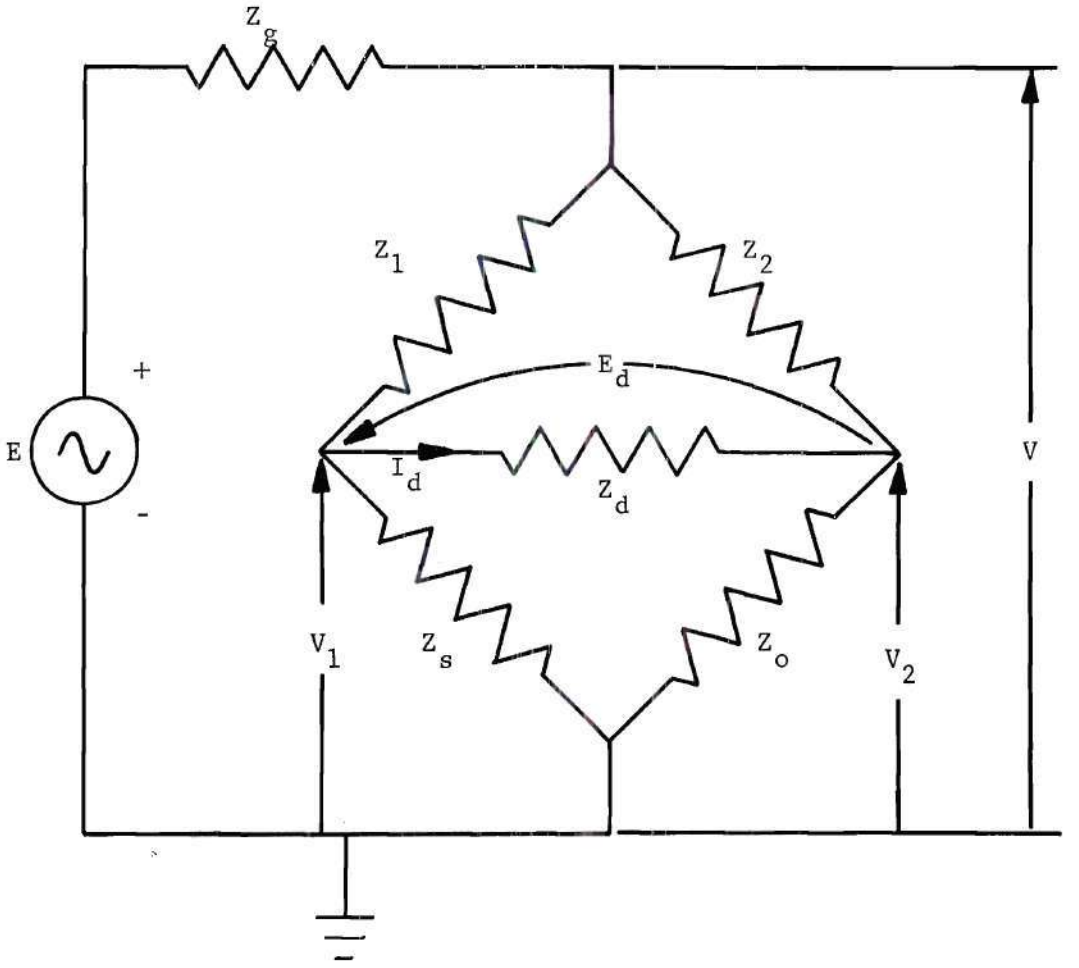


Figure A1. A Generalized Bridge Measurement System.

Therefore, by voltage division,

$$\frac{Z_s}{Z_s + Z_1} = \frac{Z_o}{Z_o + Z_2} \tag{3a}$$

which when rearranged gives,

$$\frac{Z_1}{Z_s} = \frac{Z_2}{Z_o} \tag{4a}$$

Equation(4a)will be referred to as the balance equation.

The sensitivity, S, is a function of the six bridge system components shown in Figure A1. A small change of ΔZ in the arm Z_o produces a small voltage ΔE_d which is sensed by the detector. By applying mesh analysis to the bridge in Figure A1, the detector voltage, ΔE_d, is found to be:

$$\Delta E_d = \frac{EZ_d}{(Z_o + Z_g + Z_2 + Z_g Z_2/Z_1)(Z_d + Z_s + Z_o + Z_d Z_s/Z_1)} \left[\frac{\Delta Z}{1 + K\Delta Z}\right] \tag{5a}$$

where

$$K = \frac{(Z_1)(Z_2 + Z_s) + (Z_g + Z_s)(Z_1 + Z_2 + Z_d)}{Z_1(Z_g + Z_2 + Z_o + Z_g Z_2/Z_1)(Z_d + Z_s + Z_o + Z_d Z_s/Z_1)} \tag{6a}$$

If ΔZ is very small and $K < 1$, $K\Delta Z < 1$ and can be neglected with respect to one. This restriction on K will subsequently be justified. Equation(5a)becomes:

$$\Delta E_d = \frac{Z_d E \Delta Z}{(Z_o + Z_g + Z_2 + Z_g Z_2/Z_1)(Z_d + Z_s + Z_o + Z_d Z_s/Z_1)} \tag{7a}$$

Under ideal conditions equation(7a)can be simplified further. First, as $Z_g \rightarrow 0$,

$$\Delta E_d = \frac{Z_d \, E\Delta Z}{(Z_o + Z_2)\,(Z_d + Z_s + Z_o + Z_d Z_s/Z_1)} \tag{8a}$$

Secondly, if $Z_d \rightarrow \infty$, equation(6a)becomes:

$$\Delta E_d = \frac{E\Delta Z}{(Z_o + Z_2)\,(1 + Z_s/Z_1)} \tag{9a}$$

Rearranging equation (9a) gives:

$$\frac{(\Delta E_d/E)}{(\Delta Z/Z_o)} = \frac{Z_1/Z_s}{(1 + Z_2/Z_o)\,(1 + Z_1/Z_s)} = S \tag{10a}$$

But by the balance equation (4a)

$$Z_1/Z_s = Z_2/Z_o \tag{11a}$$

By defining F to be:

$$F = Z_1/Z_s = Z_2/Z_o \,, \tag{12a}$$

equation (10a) becomes:

$$S = \frac{F}{(1 + F)^2} \tag{13a}$$

To determine maximum sensitivity, under the ideal conditions of $Z_g \rightarrow 0$ and $Z_d \rightarrow \infty$, the expression is differentiated with respect to F and set equal to zero. The maximum sensitivity occurs for F = 1, and S

is correspondingly ¼ for the case when F is a real ratio. If F is allowed to be imaginary, differentiation of the magnitude of S and solving for F shows that S is a maximum when F = j1.

To analyze the bridge in question using equation (13a) it is necessary to show that a close approximation to $Z_g = 0$ and $Z_d \rightarrow \infty$ has been realized and that for this bridge K, equation (6a) is less than one.

To show that $Z_d \rightarrow \infty$ it will be shown that $Z_d \gg Z_{eq1}$ where Z_d is the input impedance of the detector and Z_{eq1} is the effective impedance between the bridge arms as shown in Figure A2.

From Chapter IV, the values used for the bridge arm impedances and a typical value of sample impedance are:

$$Z_1 = 1.37 \text{ K } \underline{/0^\circ} \quad \text{ohms}$$

$$Z_2 = 120 \ \underline{/0^\circ} \quad \text{ohms}$$

$$Z_s = 1100 \ \underline{/-14^\circ} \quad \text{ohms}$$

$$Z_o = 96.5 \ \underline{/-14^\circ} \quad \text{ohms}$$

Since $Z_g = 3.2$ ohms is more than two orders of magnitude less than $Z_2 + Z_o$ and three orders of magnitude less than $Z_1 + Z_s$, it is neglected in the computation of Z_{eq1}:

$$Z_{eq1} = \frac{Z_1 Z_s}{Z_1 + Z_s} + \frac{Z_o Z_2}{Z_o + Z_2} \tag{14a}$$

Inserting typical values into the expression for Z_{eq1}

$$\left|Z_{eq1}\right| = 1175 \text{ ohms} \tag{15a}$$

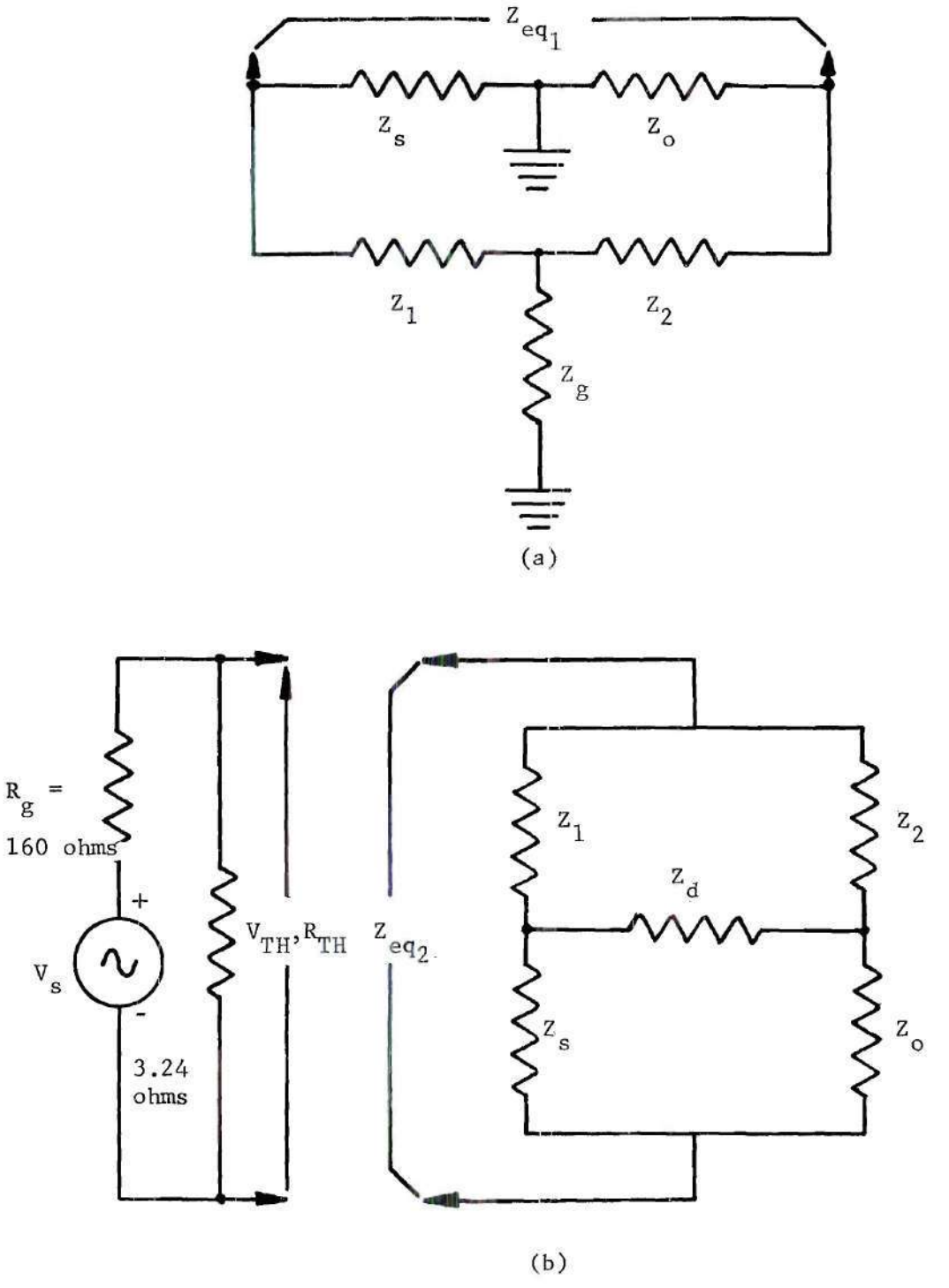


Figure A2. Equivalent Circuits for Bridge Analysis.

The input impedance of the detector is essentially the bias resistor on the gate of each buffer stage, 3.3 and 1.0 meg-ohms each. Since each resistor is better than three orders of magnitude greater than the impedances they shunt on the bridge arms, it can be reasonably assumed that they appear infinite with respect to the magnitude of Z_{eq_1}. Therefore this ideal condition for maximum bridge sensitivity is very closely approximated.

To justify the assumption that $Z_g \rightarrow 0$, it must be shown that $Z_g \ll Z_{eq_2}$, the impedance "seen" by the generator. Applying Thevenin's theorem in the manner shown in Figure A2 (b),

$$V_{TH} = \frac{3.24\ V_s}{3.24 + 160} = 0.02\ V_s \tag{16a}$$

$$R_{TH} = \frac{(3.24)\ (160)}{3.24+160} = 3.20 \text{ ohms} \tag{17a}$$

The Thevenin Resistance, R_{TH}, is the equivalent resistance of the generator, Z_g, "seen" by the load, Z_{eq_2}. Using the delta-wye transformation to simplify the circuit, Z_{eq_2} is computed to be:

$$Z_{eq_2} = \frac{Z_1\ Z_2}{Z_1+Z_2+Z_d} + \frac{\left(\frac{Z_1\ Z_d}{Z_1+Z_2+Z_d} + Z_s\right)\left(\frac{Z_2\ Z_d}{Z_1+Z_2+Z_d} + Z_o\right)}{\frac{Z_1\ Z_d}{Z_1+Z_2+Z_d} + Z_s + \frac{Z_2\ Z_d}{Z_1+Z_2+Z_d} + Z_o} \tag{18a}$$

$$Z_{eq_2} = 226\ \underline{/-47.2^\circ} \tag{19a}$$

The magnitude of Z_{eq_2} is approximately two orders of magnitude

greater than the magnitude of Z_g. Therefore, the second condition for peak bridge sensitivity is closely approximated.

It can now be shown that for the idealized conditions $Z_g \to 0$ and $Z_d \to \infty$, $K < 1$. Taking the limit of equation (6a) as $Z_g \to 0$

$$\lim_{Z_g \to 0} K = \frac{Z_1 (Z_2 + Z_s) + Z_s (Z_1 + Z_2 + Z_d)}{Z_1 (Z_2 + Z_o) (Z_d + Z_s + Z_o + Z_d Z_s / Z_1)} \tag{20a}$$

The limit of equation (20a) as $Z_d \to \infty$ is

$$\lim_{Z_d \to \infty} K = \frac{Z_s}{(Z_2 + Z_o)(Z_1 + Z_s)} \tag{21a}$$

Using the numerical values for Z_1, Z_2, Z_o, and Z_s, given on page

$$\left|K\right| = \left|2.1 \times 10^{-3} \ \underline{/-1.2^\circ}\ \right| < \left|\ 1.0 \ \underline{/0^\circ}\ \right|$$

The circuit diagram for the detector is shown in Figure A3. Two FET source-follower stages are used for isolation, and to drive a differential amplifier. The buffer stages provide a high impedance to the bridge while supplying a low-impedance driving source for the differential amplifier inputs.

The two FET stages can be analyzed by using equations developed in Millman and Halkias[28] for generalized source-follower amplifiers. For frequencies where the coupling capacitors may be considered shorts (their reactance at 1 kHz is less than 4 ohms) the gain of the stage is given by:

$$A_v = \frac{g_m R_s}{1 + (g_m + g_d) R_s} \tag{22a}$$

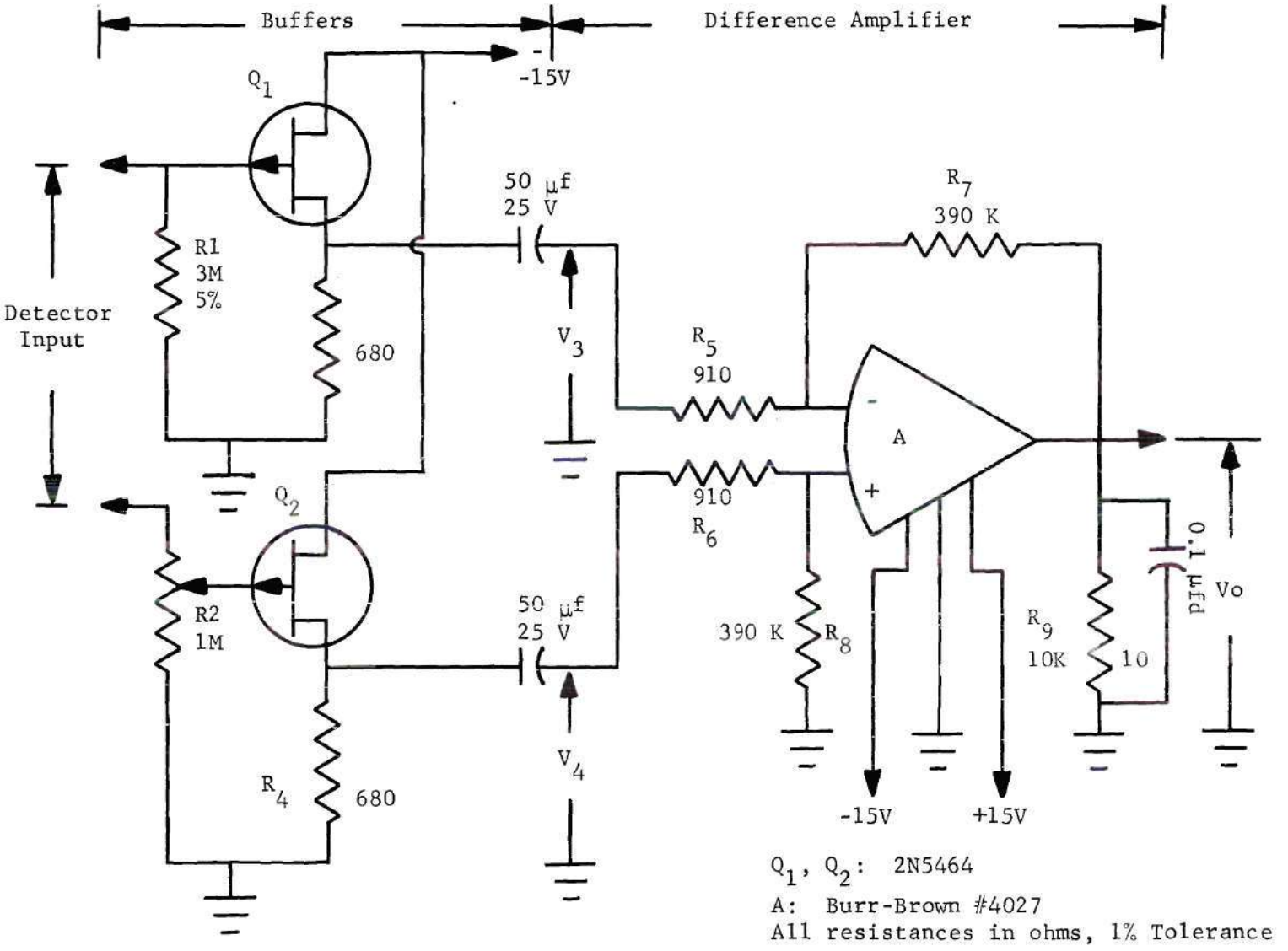


Figure A3. Bridge Detector Schematic.

where

g_m = transconductance of the FET

g_d = source to drain conductance of the FET

R_s = source resistor value

The substitution of typical values for the 2N5464 FET (g_m = 3000 μmhos, g_d = 50 μmhos) and the source resistor in Figure A3 into equation (22a) gives:

$$A_v = 0.665$$

To analyze the difference amplifier section of Figure A3, two voltages are defined:

$$V_d = V_4 - V_3 \tag{23a}$$

$$V_c = \tfrac{1}{2}(V_3 + V_4) \tag{24a}$$

where V_d is the difference-mode voltage and V_c is the common-mode voltage. It is desired to find V_o the output voltage as a function of V_d and V_c, that is:

$$V_o = A_d V_d + A_c V_c \tag{25a}$$

where A_d and A_c are functions of the circuit parameters and

$$A_d = V_o/V_d \Big|_{V_c = 0} \qquad A_c = V_o/V_c \Big|_{V_c = 0}$$

Superposition is used to find V_o as a function of V_3 and V_4:

$$V_o \Big|_{V_4 = 0} = V_3(-R_7/R_5)$$

$$V_o \Big|_{V_3 = 0} = \frac{R_8}{(R_8 + R_6)} \frac{(R_5 + R_7)}{R_5}$$

Therefore:

$$V_o = \frac{-R_7}{R_5} V_3 + \frac{R_8 (R_5 + R_7)}{R_5 (R_8 + R_6)} V_4 \qquad (26a)$$

To find A_d, V_c is set equal to zero, which gives:

$$V_c = 0 = \tfrac{1}{2}(V_4 + V_3) \Longrightarrow V_4 = - V_3 \qquad (27a)$$

Substitution into equation (26a) yields:

$$\frac{V_o}{V_3} = \frac{-R_7(R_8 + R_6) - R_8(R_5 + R_7)}{R_5(R_8 + R_6)} \qquad (28a)$$

Since

$$V_d = V_4 - V_3 \qquad (29a)$$

and from (27a)

$$V_4 = -V_3 \qquad (30a)$$

equation (28a) becomes

$$V_d = -2\,V_3 \quad \text{or} \quad V_3/V_d = -\tfrac{1}{2} \tag{31a}$$

Also,

$$V_o/V_d \Big|_{V_c = 0} = V_o/V_3 \times V_3/V_d = \tfrac{1}{2}\,\frac{R_7(R_8 + R_6) = R_8(R_5 + R_7)}{R_5(R_8 + R_6)} \tag{32a}$$

Therefore

$$A_d = \tfrac{1}{2}\,\frac{R_7(R_8 + R_6) + R_8(R_5 + R_7)}{R_5(R_8 + R_6)} \tag{33a}$$

To make $V_d = 0$, set $V_4 = V_3$ then substituting into equation (26a) gives:

$$V_o/V_3 = \frac{R_8R_5 - R_7R_6}{R_5(R_8 + R_6)} \tag{34a}$$

Again since

$$V_c = \tfrac{1}{2}\,(V_3 + V_4) = \tfrac{1}{2}\,(V_3 + V_3) = V_3 \tag{35a}$$

then

$$V_3/V_c = 1 \tag{36a}$$

and

$$(V_o/V_3) \times (V_3/V_c) = V_o/V_c \Big|_{V_d = 0} = \frac{R_8R_5 - R_7R_6}{R_5(R_8 + R_6)} \tag{37a}$$

Hence

$$A_c = \frac{R_8R_5 - R_6R_7}{R_5(R_8 + R_6)} \tag{38a}$$

The common-mode amplification is undesirable in that it prevents nulling the output voltage completely to zero. Even if $V_3 = V_4$ and $V_d = 0$ there will be an output potential because

$$V_o = A_c\ (V_3 + V_4)/2 \ + \ A_d\ (0) \tag{39a}$$

To avoid this problem A_c can be made zero of

$$R_8R_5 \ = \ R_6R_7 \tag{40a}$$

By choosing in Figure A3

$$R_5 = R_6 = 910 \text{ ohms}$$

$$R_7 = R_8 = 390 \text{ K ohms}$$

$$R_9 \ = 10 \text{ K ohms}$$

$$R_3 = R_4 = 680 \text{ ohms}$$

equation (12a) is satisfied making $A_c = 0$ and

$$A_d \ = \ \frac{(390K)\ (390K + 910) + (390K)\ (390K + 910)}{2\ (1590)\ (390K + 910)} \tag{41a}$$

$$A_d \ = \ 408 \tag{42a}$$

Multiplying this by the buffer amplifier gain, the detector gain G_d is:

$$G_d = 408 \text{ X } 0.66 = 275$$

Having justified the use of equation (13a) by showing that the ideal conditions associated with it are closely approximated, and having found an expression for the gain of the detector, equation (13a) is rearranged to solve for $\Delta Z/Z_o$ giving

$$\Delta Z/Z_o = (1/S) \text{ X } (\Delta E_d/E) \tag{43a}$$

The Tektronix 585A oscilloscope and type 82 plug-in unit can easily detect a 10 mV voltage signal. This would mean that the maximum difference voltage which could exist undetected across the input to the detector is:

$$\Delta E_d(\text{max}) = 10 \text{ mV}/275 \cong 0.036 \text{ mV} \tag{44a}$$

Using the previously stated values for typical impedances of the bridge arms and the maximum value of ΔE_d, equation (20a) becomes:

$$\Delta Z/Z_o = (3.88)\ (0.036)/(100) = 0.0014 = 0.14\% \tag{45a}$$

Thus, the theoretical errors due to the sensitivity of the bridge are less that 0.2 per cent.

Readout Errors

The resistive standard, R_s is a 2 K ohm ten-turn helipot with vernier drive readable to three significant figures. Therefore, the readability is 1/1000 or $\pm$ 0.1 per cent.

The capacitive standard employed was made up of two decade capacitances between them. A switching arrangement allowed readout to 1

part in 100 or 1 per cent. Since exact balance was seldom achieved because the capacitance standard is not continuously variable over its range, the last digit may be in error by ± 1 unit, reducing the accuracy to 1 part in 50 or 2 per cent.

Summary

It has been shown that errors in bridge sensitivity are less than 0.2 per cent and that readout errors contribute 0.1 per cent error on the resistive part and 2 per cent on the reactive part. Therefore, bounds can be set on the impedance measurement error:

Source	Readout	Sensitivity	Total
Resistive error	0.1 per cent	0.2 per cent	0.3 per cent
Reactive error	2.0 per cent	0.2 per cent	2.2 per cent

The focus of this work deals with the resistive part of the impedance and the accuracy (0.3 per cent) will be used to interpret the results of the experiment. Any changes in the resistance must be greater than 0.3 per cent of the magnitude of the resistive component if one is to be certain such a change occurs. A shift of at least an order of magnitude (± 3 per cent) would be desirable.

The reactive part must be interpreted with even more caution. The 2.2 per cent error will probably preclude anything more than a qualitative interpretation.

APPENDIX B

COMMERCIAL EQUIPMENT USED IN THE EXPERIMENT

This text contains technical specifications on the major items of commercial equipment used.

Oscilloscope: Tektronix Type 585A with type 82 dual-trace plug-in. Horizontal sweep speed set at 1 μsec/cm, vertical sensitivity set at 5 mV/cm and X 10 probe used making net vertical sensitivity 50 mV/cm.

Audio Oscillator: Hewlett-Packard type 200CD. Output impedance at 1 kHz measured to be 160 ohms.

Power Supplies: Hewlett-Packard type 721 solid-state supply. Outputs set at ± 15 volts.

Water Bath: Precision Scientific Type 6648. Capacity approximately 12 quarts. Thermostat set for 37° C temperature.

Thermometer: J. W. Will type 26801. Range -20° to 110° C.

Operational Amplifier: Burr-Brown type 3077/12C.

Field Effect Transistors: Motorola type 2N5464

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